Increased physical protection of soil carbon in the mineral soil of a poplar plantation after five years of free atmospheric CO2 enrichment (FACE)

M. R. Hoosbeek, J. M. Vos, G. E. Scarascia-Mugnozza

Biogeosciences Discuss., 3, 871–894, 2006
www.biogeosciences-discuss.net/3/871/2006/

Biogeosciences Discussions

Biogeosciences Discussions is the access reviewed discussion forum of *Biogeosciences*

Increased physical protection of soil carbon in the mineral soil of a poplar plantation after five years of free atmospheric CO_2 enrichment (FACE)

M. R. Hoosbeek[1], **J. M. Vos**[1], **and G. E. Scarascia-Mugnozza**[2]

[1]Wageningen University, Department of Environmental Sciences, Earth System Science group, P.O. Box 37, 6700AA Wageningen, The Netherlands
[2]University of Tuscia, Department of Forest Environment and Resources, Via S.Camillo De Lellis, 01100 Viterbo, Italy

Received: 24 May 2006 – Accepted: 7 June 2006 – Published: 5 July 2006

Correspondence to: M. R. Hoosbeek (marcel.hoosbeek@wur.nl)

Abstract

Free air CO_2 enrichment (FACE) experiments in aggrading forests and plantations have demonstrated significant increases in net primary production (NPP) and C storage in forest vegetation. The extra C uptake may also be stored in forest floor litter and in forest soil. After five years of FACE treatment at the EuroFACE short rotation poplar plantation, the increase of total soil C% was larger under elevated than under ambient CO_2. However, the fate of this additional C allocated belowground remains unclear. The stability of soil organic matter is controlled by the chemical structure of the organic matter and the existence of protection offered by the soil matrix and minerals. Fresh litter entering the soil enhances microbial activity which induces the binding of organic matter and soil particles into macro-aggregates. As the enclosed organic matter is decomposed, microbial and decomposition products become associated with mineral particles. This association results in the formation of micro-aggregates (within macro-aggregates) in which organic matter is stabilized and protected. FACE and N-fertilization treatment did not affect the micro- and macro-aggregate weight, C or N fractions obtained by wet sieving. However, *Populus euramericana* increased the micro- and small macro-aggregates weight and C fractions. The obtained macro-aggregates were broken up in order to isolate recently formed micro-aggregates within macro-aggregates (iM-micro-aggregates). FACE increased the iM-micro-aggregate weight and C fractions. This study reveals that: 1) Species has an effect on the formation of macro-aggregates. The choice of species in a plantation or the effect of global change on species diversity, may therefore affect the stabilization and protection of soil C in aggregates. And 2) Increased atmospheric CO_2 concentration increases the stabilization and protection of soil C in micro-aggregates formed within macro-aggregates. This mechanism increases the C sink of forest soils under increasing atmospheric CO_2 concentration.

BGD

3, 871–894, 2006

1 Introduction

Afforestation of agricultural land and regrowth of temperate forests constitute a large carbon (C) sink (Houghton, 2003; Houghton et al., 1998; Janssens et al., 2003). Enhanced growth due to increasing atmospheric CO_2 concentration is hypothesized to further increase this terrestrial C sink (Prentice et al., 2001). Free air CO_2 enrichment experiments in aggrading forests and plantations have demonstrated significant increases in net primary production (NPP) and C storage in forest vegetation (Calfapietra et al., 2003; DeLucia et al., 1999; Gielen et al., 2005; Hamilton et al., 2002; Liberloo et al., 2006; Norby et al., 2005; Norby et al., 2002). The extra C uptake may, next to forest vegetation, also be stored in forest floor litter and in forest soil. The fate of this additional C allocated belowground remains unclear (Jastrow et al., 2005; Lichter et al., 2005; Norby et al., 2002; Schlesinger and Lichter, 2001). Enhanced carbon transfer to the root system may result mainly in enhanced root respiration or, otherwise, in an increase of root dry matter, mycorrhizal activity and subsequent transfer of carbon to soil C pools.

The stability of soil organic matter is controlled by the chemical structure of the organic matter and the existence of protection offered by the soil matrix and minerals (Baldock and Skjemstad, 2000; Davidson and Janssens, 2006; Elliott, 1986; Jastrow, 1996; Krull et al., 2003; Six et al., 2002; Van Veen and Kuikman, 1990). The additional C input into the soil may affect population size and activity of soil fauna and flora, and may therefore also affect the formation of soil aggregates (Oades, 1993). It has been established that the inclusion of organic matter within aggregates reduces its decomposition rate (Krull et al., 2003; Oades, 1984; Six et al., 2002, 2000; Tisdall and Oades, 1982). Oades (1984, 1993) suggested a model of aggregate formation in which micro-aggregates (~100 μm in diameter) are formed within macro-aggregates (>250 μm in diameter). This model of the *cycle of aggregate formation* has been extended and applied by Jastrow (1996), Puget (1995) and Six et al. (2002, 2001, 1999, 1998). Fresh plant remains entering the soil become sites for microbial activity and nu-

cleation centers for aggregation. The enhanced microbial activity induces the binding of organic matter and soil particles into macro-aggregates. As the enclosed organic matter is decomposed, microbial and decomposition products become associated with mineral particles (Chenu and Stotzky, 2002). This association results in the formation of micro-aggregates. Eventually, the binding agents in macro-aggregates degrade, resulting in a breakdown of macro-aggregates and the release microbially processed organic matter and micro-aggregates. Six et al. (1999) hypothesized that these released micro-aggregates are a mixture of old micro-aggregates, which had been previously formed and incorporated during macro-aggregate formation, and newly formed micro-aggregates.

At two sites in Ohio and Ontario Six et al. (2002) found that afforestation of cultivated land resulted in increased aggregation and a greater C stock in the A horizons. The micro-aggregates and their capacity to protect C in the longer term were found to be crucial for C sequestration in both forested systems. Twenty years after afforestation of former arable land in northeastern Italy, Del Galdo et al. (2003) observed an increase of soil C of respectively 23 and 6% at 0–10 and 10–30 cm soil depth. Moreover, afforestation resulted in stabilization of soil C in micro-aggregates.

After six years of CO_2 enrichment at the Duke Forest FACE experiment, Lichter et al. (2005) did not detect a significant FACE effect on soil C content. However, the C content of the mineral top soil (0–15 cm) averaged over the FACE and control rings significantly increased during the experiment. Physical fractionation suggested that this increase occurred entirely within the free light fraction in which organic C is not protected against decomposition. Fractions in which soil C is protected to some degree, i.e. coarse and fine intra-aggregate particulate organic matter (iPOM) and mineral associated organic matter (micro-aggregates) were not affected by FACE. At the Oak Ridge deciduous forest FACE experiment, organic C in the surface 5 cm of the soil increased linearly during 5 years of CO_2 enrichment, while C in the ambient plots remained relatively constant (Jastrow et al., 2005).

After 3 years of FACE treatment at the POPFACE poplar plantation, significantly

more new C was incorporated into the mineral soil (Hoosbeek et al., 2004). We also observed a significantly larger increase in total soil C after 5 years of CO_2 enrichment (Hoosbeek et al., 2006). Chemical fractionation revealed a FACE induced increase of the labile soil C fraction. We hypothesize that this extra labile soil C will increase the formation of macro-aggregates and subsequently will increase the formation of micro-aggregates. This FACE enhanced physical protection of soil organic matter may increase long-term C sequestration in forest soils.

2 Methods

The POPFACE experiment was established early 1999 on former agricultural fields near Viterbo (42°37′04″ N, 11°80′87″ E, alt 150 m), Italy. The plantation and adjacent fields had been under forest until about 1950. Since then a variety of agricultural crops has been grown on these former forest soils until the inception of the POPFACE plantation. The annual precipitation is on average 700 mm with dry summers (Xeric moisture regime). During November of 1998 an initial soil survey took place. The loamy soils classified as Pachic Xerumbrepts and were described in detail by Hoosbeek et al. (2004).

Nine ha were planted with *Populus x euramericana* hardwood cuttings at a density of 0.5 trees per m^2. Within this plantation three FACE and three control plots (30×30 m) were randomly assigned under the condition of minimum CO_2 enrichment pollution. The plots were divided into two parts by a physical resin-glass barrier (1 m deep in the soil) for nitrogen differential treatments in the two halves of each plot. However, because of the high inorganic N content of the soil, no fertilization treatment was applied during the first 3-year rotation of the experiment. Each half plot was divided into three sectors, where each sector was planted at a density of 1 tree per m^2 using three different genotypes: *P. x euramericana* Dode (Guinier) (=*P. deltoides* Bart. ex Marsh. x *P. nigra* L.) genotype I-214, a genotype of *P. nigra* L. (Jean Pourtet) and a local selection of *P. alba* L. (genotype 2AS11). Carbon enrichment was achieved by injection of pure

CO_2 through laser-drilled holes in tubing mounted on six masts (Miglietta et al., 2001). The FACE rings (octagons) within the FACE plots had a diameter of about 22 m. The elevated CO_2 concentrations, measured at 1-min intervals, were within 20 % deviation from the pre-set target concentration (560 μmol mol^{-1}) for 91% of the time to 72.2% of the time, respectively, at the beginning and at the end of each rotation cycle of the plantation. The plantation was drip irrigated at a rate of 6 to 10 mm per day during the growing seasons.

The trees were coppiced after the first three growing seasons (1999–2001). The experiment continued with a second rotation under the name EuroFACE (2002–2004). A fertilization treatment was added to one half of each experimental plot because soil analyses showed the occurrence of limiting conditions of nitrogen availability in the soil (Scarascia-Mugnozza et al., 2006). The total amount of nitrogen supplied was 212 kg ha^{-1} y^{-1} in 2002 and 290 kg ha^{-1} y^{-1} during 2003 and 2004.

Soil samples were collected from each sector within 2 control (rings 2 and 3) and 2 FACE plots (rings 1 and 4) in October of 2003. Bulk density samples were taken with 300 cm^3 metal rings at 0–10 cm below the surface of the mineral soil. The samples were dried at 105°C for 3 days. Bulk densities were calculated based on dry weight and ring volume. Next, the soil samples were crushed by hand and live roots were removed. Carbon and nitrogen were determined by flash combustion in an elemental analyzer (EA 1108) (Van Lagen, 1996). Total soil organic C and N content are expressed as weight percentage (g C or N per gram soil × 100%).

2.1 Whole sample fractionation

For fractionation, bulk samples were collected from the upper 10 cm of the mineral soil and air dried at room temperature. Before drying, large aggregates (>1 cm) were broken up along natural planes of weakness. The wet sieving procedure was described by Six et al. (1998). Materials used included a wet sieving apparatus, 20 l buckets (used as wet sieving basins) and four 20 cm diameter sieves (2000, 1000, 250 and 53 μm mesh). The buckets were filled with demineralized water; the sieves were stacked,

submerging one sieve at a time to prevent air bubbles from getting trapped under a sieve. The top sieve (2000 μm) was placed on top of the stack without touching the water at first. Dried soil material was placed on the top sieve, after which the stack of sieves was lowered until the material on the top sieve was just covered by water. The samples were left to slake for 5 min, followed by 2 min of wet sieving. The wet sieving apparatus gently lowers and lifts the sieves at a speed of about 30 repetitions per minute, over a distance of 3 cm. After sieving, the sieves were lifted out of the water and the material that remained on the sieves was washed into beakers assigned to the specific fractions. The isolated fractions were dried at 40°C. Four fractions based on the following size classes were distinguished: 53–250 (micro-aggregates), 250–1000 (small macro-aggregates), 1000–2000 (medium macro-aggregates), and >2000 μm (large macro-aggregates).

Six et al. (1998) used three sieves: 2000, 250 and 53 μm, while we used an additional 1000 μm sieve. The reason for this was the relatively large 250–2000 μm fraction of the samples we used, which tended to block the 250 μm sieve. We also used 75 g of sample per stack of sieves instead of 100 g in order to prevent blockage.

In general, differences in texture between field plots and aggregate fractions, and the fact that there is hardly any binding between sand particles and organic matter, makes sand fraction correction a necessity when comparing aggregate fractions and their C and N contents (Elliott et al., 1991; Six et al., 1998). Per obtained fraction, 2 gram of soil material was used. The aggregates were destroyed by removing organic matter with peroxide and dispersing clay with a dispersing solution of sodium carbonate and sodium polyphosphate. Dispersion was completed by ultrasonic treatment (Van Doesburg, 1996). Next, the destroyed size fraction was washed over the original sieve. The material left on the sieve was taken as the sand fraction. Aggregate weight fractions were calculated as:

$$\text{Aggregate weight fraction}_{(1..4)} = \frac{\text{total weight fraction}_{(1..4)} - \text{weight sand fraction}_{(1..4)}}{\text{total sample weight}}$$

C and N contents could only be determined for uncorrected fractions (because the

sand correction includes the loss of organic material). However, we assumed that the sand fractions of the aggregate fractions and total sample did not contain C or N (just inert minerals). The aggregate C fractions were calculated as:

$$\text{Aggregate C fraction}_{(1..4)} = \frac{\text{g C fraction}_{(1..4)}}{\text{g C total sample}}$$

The aggregate N fractions were calculated in a similar fashion.

2.2 Intra-macro-aggregate (iM) fractions

Next, the small en medium sized macro-aggregate fractions were combined into one 250–2000 μm macro-aggregate fraction. Breaking up this macro-aggregate fraction will, according to the aggregate formation model, result in the release of clay and silt (<53 μm), micro-aggregates (53–250 μm), and course POM and sand (>250 μm). These intra-macro-aggregate fractions will be indicated by "iM". A "micro-aggregate isolator", as described by Six et al. (2002), was used to break up the macro-aggregates while minimizing the break down of the released iM-micro-aggregates. Ten grams of macro-aggregates were immersed in deionized water on top of a 250 μ mesh screen and shaken with 50 glass beads (4 mm diameter). A continuous water flow through the device flushed all released iM-micro-aggregates immediately onto a 53 μm sieve, thus avoiding further disruption. After complete breakup of the macro-aggregates, coarse iM-POM and sand remained on the 250 μm mesh screen. The iM-micro-aggregates and the iM-clay and silt fraction were separated by the 53 μm sieve. Weight, C and N fractions were calculated as mentioned above.

2.3 Statistics

The SPSS (v 11.5) General Linear Model was used to calculate univariate analysis of variance and to evaluate FACE and N-fertilization treatment and species effects. Differences between means were considered significant when the P-value of the UNIANOVA

F-test was <0.05.

3 Results

At the end of the fifth growing season, average soil C and N percentages of the top 10 cm of the mineral soil in the sampled rings (1–4) were 1.19 and 0.12% respectively (Table 1). The average bulk density was 1.22 g soil $\cdot$ cm^{-3}.

3.1 Whole sample fractionation

The small macro-aggregate (250–1000 μm) weight fraction was the largest fraction making up one-third of the total sample weight (Table 2). The second largest weight fraction was the large macro-aggregate (>2000 μm) fraction. FACE and N fertilization treatments had no effect on aggregate weight fractions. However, differences in species did change the weight distribution among the aggregate fractions significantly. *Populus euramericana*, as compared to *P. alba* and *nigra*, increased the micro-aggregate (53–250 μm) and small macro-aggregate fractions and decreased the large macro-aggregate fraction.

Based on C content, the small macro-aggregate aggregate fraction was again the largest holding on average 44% of the carbon (Table 3). FACE and N fertilization treatments had no effect on the C distribution. However, *P. euramericana* increased the small macro-aggregate C fraction while it decreased the large macro-aggregate C fraction. These trends were close to being significant (0.05<P<0.10).

Most N was contained in the small macro-aggregate fraction, making up almost one-third of the total (Table 4). Again, only a species effect was observed. *P. euramericana* decreased the medium (1000–2000 μm) and large macro-aggregate N fractions.

BGD
3, 871–894, 2006

EGU

3.2 Intra-macro-aggregate (iM) fractions

The combined small and medium macro-aggregates consisted of, on average by weight percentage, 57% iM-micro-aggregates, 24% iM-coarse POM and sand, and 15% iM-clay and silt particles. FACE and N fertilization treatments and poplar species had no significant effect on the iM-aggregate weight fractions. Although, FACE did increase the iM-micro-aggregate weight fraction, i.e. 0.60 (FACE) vs. 0.53 (ambient) with limited significance (P=0.104) (Table 5).

FACE did, however, significantly increase the iM-micro-aggregate C fraction. FACE did not affect the iM-clay and silt and iM-coarse POM and sand C fractions (data not shown). N treatment and poplar species had no effect on the iM-C fractions.

The iM-N fractions were not affected by either FACE or N treatment. *Populus euramericana*, however, did increase the iM-micro-aggregate N fraction as compared to *P. alba* and *nigra*.

4 Discussion

In preparation of the POPFACE experiment the experimental field was ploughed during the Fall of 1998 (Hoosbeek et al., 2004). At that time the structure of the loamy A horizon was characterized by coarse prismatic peds (FAO, 1990). Five years after establishment of the plantation, three litter layers (L, F and H) had formed on top of the mineral soil. Underneath, the structure of the A horizon had changed into fine and medium sized blocky and granular aggregates. In the F, H and A horizons many arthropods and few earthworms were observed. Afforestation obviously changed the structure of the top soil within 5 years.

We hypothesized that FACE treatment, through an increase of net primary production and increased C input into the soil, would increase the formation of macro-aggregates and subsequently the formation of micro-aggregates. We also expected N-fertilization to have a positive effect on aggregate formation because of increased N availability to

plants and soil flora and fauna.

4.1 Whole sample fractionation

The results of whole sample fractionation showed no FACE or N fertilization effect on aggregate formation. Instead, a species effect was observed. Fewer large sized and more small sized macro-aggregates were present under *P. euramericana*. Moreover, *P. euramericana* increased the formation of micro-aggregates. According to the model of aggregate formation, in which micro-aggregates are formed within macro-aggregates, this increase of micro-aggregates under *P. euramericana* may be explained in two ways: 1) More large sized macro-aggregates broke up prior to sampling which increased the number of released micro-aggregates. 2) More small sized macro-aggregates are being formed and subsequently more micro-aggregates are being released. Explanation 1 assumes increased disruption or degradation of large macro-aggregates under *P. euramericana*. Also, it assumes that the large macro-aggregate fraction was not replenished by newly formed large macro-aggregates. Explanation 2 assumes an increase in the speed of the cycle of aggregate formation, resulting in an increase of macro-aggregate formation and subsequently an increase of micro-aggregate formation. Explanation 1 seems unlikely because there is no good reason for increased disruption of large macro-aggregates and the subsequent lack of formation of new large sized macro-aggregates under *P. euramericana*. Explanation 2 is supported by estimates of fresh plant remains entering the soil. Lukac et al. (2003) calculated the amount of C transferred into the soil via fine roots during the first rotation (POPFACE) as a result of fine root production and turnover. They estimated the C input for respectively *P. alba*, *nigra* and *euramericana* under ambient CO_2 to be: 124, 128 and 170 (g/m^2); and under FACE respectively: 189, 232 and 309 (g/m^2). The actual amount of C entering the soil was thought to be higher because root respiration and exudation was not accounted for. However, on average, under ambient CO_2 and FACE, 42% more C entered the soil via fine roots under *P. euramericana* as compared to under *alba* and *nigra*. Hoosbeek et al. (2004) estimated the input of C into the soil

with the C3/C4 stable isotope method during the second and third year. Again, most C entered the soil under *P. euramericana*, i.e. on average 16 and 15% more new C in respectively 2000 and 2001 as compared to *P. alba* and *nigra*. The observed larger C inputs and increase of small macro-aggregate and micro-aggregate fractions under *P. euramericana* are in agreement with the *cycle of aggregate formation* model. The larger input of fresh litter under *P. euramericana* increased the number of sites for microbial activity and the number of nucleation centers for aggregation. Enhanced microbial activity induced an increase of binding of organic matter and soil particles into macro-aggregates. More enclosed organic matter was decomposed and was subsequently associated with mineral particles. Increased association resulted in increased formation of micro-aggregates and long term stabilization of soil organic matter.

As with the weight fractions, the large and medium macro-aggregate C fractions decreased, and the small macro-aggregate C fraction increased under *P. euramericana*. However, the micro-aggregate C fraction did not significantly increase under *P. euramericana*. Elliott (1986), Gupta and Germida (1988) and Six et al. (2000) also found that macro-aggregates contain relatively more C than micro-aggregates. Elliott (1986) regarded the organic matter in macro-aggregates to be highly susceptible to mineralization and found the C in micro-aggregates to be more recalcitrant. Under *P. euramericana* more fresh plant C was incorporated into small macro-aggregates. During decomposition part of the C was lost as CO_2 from the macro-aggregates and another part was associated with mineral parts contributing to the formation of micro-aggregates. This loss of CO_2 and formation of micro-aggregates decreases the C to mineral particles weight ratio. This may explain why the micro-aggregate weight fraction significantly increased under *P. euramericana*, while the micro-aggregate C fraction also increased but not significantly.

Like the weight and C fractions, the large and medium macro-aggregate N fractions decreased under *P. euramericana*. A species effect on the small macro-aggregate and micro-aggregate N fractions was probably obscured by the relatively high C/N ratio of the fresh plant material as compared to the lower C/N ratio of the older organic

matter included in the macro-aggregates. Likewise, the decrease of C/N ratio during decomposition and humification probably caused an absence of a species effect on the micro-aggregate N fraction.

4.2 Intra-macro-aggregate (iM) fractions

The isolated iM-micro-aggregate weight fraction was larger under FACE than under ambient CO_2, although with limited significance (Table 5; P=0.104). However, based on C, the iM-micro-aggregate fraction was significantly (P=0.003) larger under FACE. These results may seem to contradict the fact that the small and medium macro-aggregate fractions, that harbored these iM-micro-aggregate fractions, were not affected by FACE. However, the fractions remaining on the sieves after wet sieving represent aggregate fractions with a certain minimum aggregate stability. Similar weight fractions may still include aggregates with different stabilities above a certain minimum. Although the small and medium macro-aggregate fractions were not affected by FACE, the average stability of the small and medium macro-aggregate fractions formed under FACE may have been higher. A higher stability causes a slower macro-aggregate turnover, which enhances the formation of iM-micro-aggregates inside (Six et al., 2000). So, after five years of treatment, FACE increased the formation of the iM-micro-aggregate C fraction probably due to increased stability of small and medium macro-aggregates. Eventually, these iM-micro-aggregates will be released from the "nursery" and increase the "free" micro-aggregate C fraction as fraction of the whole soil. Through this mechanism, FACE increases the stabilization and protection of soil C in iM-micro-aggregates, as shown by our results, and will eventually, as we expect, also increase the stabilization and protection of soil C in "released" micro-aggregates.

4.3 Forest FACE experiments

Until recently, increased atmospheric CO_2 concentration was reported to have no significant effect on soil C sequestration at forest FACE experiments (Houghton, 2003;

Lichter et al., 2005; Norby et al., 2002; Schlesinger and Lichter, 2001). After six years of CO_2 enrichment at the Duke Forest FACE experiment, Lichter et al. (2005) did not detect a significant FACE effects on soil C content. However, the C content of the mineral top soil (0–15 cm) averaged over the FACE and control rings significantly increased during the experiment due to regrowth. Physical fractionation suggested that this increase occurred entirely within the free light fraction in which SOM is not protected against decomposition. The iPOM and iM-micro-aggregate C fractions were not affected by FACE. Lichter et al. (2005) concluded that forest soils are unlikely to sequester significant additional quantities of atmospheric C associated with CO_2 fertilization because of the low rates of C input to refractory and protected SOM pools.

Recently, Jastrow et al. (2005) raised the question whether the lack of a FACE effect on soil C content is a general response or a function of (1) the low statistical power of most experiments, and/or (2) the magnitude of CO_2-stimulated C inputs relative to the duration of the experiments. At the Oak Ridge deciduous forest FACE experiment, organic C in the surface 5 cm of the soil increased linearly during 5 years of CO_2 enrichment, while C in the ambient plots remained relatively constant (Jastrow et al., 2005). A significant FACE effect on soil C was observed for the top 5 cm. Sampling of a thicker soil increment, e.g. 0–15 cm, would have "diluted" the increase of C which would have resulted in a non-significant effect. A meta-analysis of 35 independent experimental observations from a wide range of ecosystems showed that CO_2 enrichment increased soil C by 5.6% (Jastrow et al., 2005). According to Jastrow et al. (2005), this result supports the generality of the observed increase of soil C under FACE at the Oak Ridge experiment.

At the EuroFACE site we also observed a significant larger increase in total soil C% after 5 years of CO_2 enrichment (Hoosbeek et al., 2006). Chemical fractionation revealed that this increase occurred within the labile soil C fraction. The question remained whether the observed larger increase of total soil C and the increase of labile soil C under FACE would eventually result in long-term C sequestration in stable organic matter fractions. The results of physical fractionation presented in this study

reveal that:

1. Species has an effect on the formation of macro-aggregates. The choice of species in a plantation or the effect of global change on species diversity, may therefore affect the stabilization and protection of soil C in aggregates.

2. Increased atmospheric CO_2 concentration increases the stabilization and protection of soil C in micro-aggregates being formed within macro-aggregates. This mechanism increases the C sink of forest soils under increasing atmospheric CO_2 concentration.

Acknowledgements. We thank J. Marinissen for her advice and help with the wet sieving and sand fraction procedures. C. Calfapietra and colleagues are gratefully acknowledged for managing and maintaining the FACE facility. Funding was provided by the European Commission Fifth Framework Program, Environment and Climate RTD Program, research contract EVR1-CT- 2002-40027 (EUROFACE) and by the Centre of Excellence "Forest and Climate" of the Italian Ministry of University and Research (MIUR).

References

Baldock, J. A. and Skjemstad, J. O.: Role of the soil matrix and minerals in protecting natural organic materials against biological attack, Organic Geochemistry, 31, 697–710, 2000.

Calfapietra, C., Gielen, B., Galema, A. N. J., Lukac, M., De Angelis, P., Moscatelli, M. C., Ceulemans, R., and Scarascia-Mugnozza, G.: Free-air CO_2 enrichment (FACE) enhances biomass production in a short-rotation poplar plantation, Tree Physiology, 23, 805–814, 2003.

Chenu, C. and Stotzky, G.: Interactions between microorganisms and soil particles: An overview, in: Interactions between microorganisms and soil particles, edited by: Huang, P. M., Bollag, J.-M., Senesi, N., John Wiley & Sons, Ltd, 2002.

Davidson, E. A. and Janssens, I. A.: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change, Nature, 440, 165–173, 2006.

Del Galdo, I., Six, J., Peressotti, A., and Cotrufo, M. F.: Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes, Global Change Biol., 9, 1204–1213, 2003.

DeLucia, E. H., Hamilton, J. G., Naidu, S. L., Thomas, R. B., Andrews, J. A., Finzi, A. C., Lavine, M., Matamala, R., Mohan, J. E., Hendrey, G. R., and Schlesinger, W. H.: Net primary production of a forest ecosystem with experimental CO_2 enrichment, Nature, 284, 1177–1179, 1999.

Elliott, E. T.: Aggregate structure and carbon, nitrogen, and phosphorous in native and cultivated soils, Soil Sci. Soc. A. J., 50, 627–633, 1986.

Elliott, E. T., Palm, C. A., Reuss, D. E., and Monz, C. A.: Organic matter contained in soil aggregates from a tropical chronosequence: Correction for sand and light fraction, Agric. Ecosyst. Environ., 34, 443–451, 1991.

FAO: Guidelines for soil description, Food and Agricultural Organization of the United Nations, Rome, 1990.

Gielen, B., Calfapietra, C., Lukac, M., Wittig, V. E., De Angelis, P., Janssens, I. A., Moscatelli, M. C., Grego, S., Cotrufo, M. F., Godbold, D. L., Hoosbeek, M. R., Long, S. P., Miglietta, F., Polle, A., Bernacchi, C. J., Davey, P. A., Ceulemans, R., and Scarascia-Mugnozza, G. E.: Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO_2 enrichment, Tree Physiology, 25(11), 1399–1408, 2005.

Gupta, V. V. S. R. and Germida, J. J.: Distribution of microbial biomass and its activity in different soil aggregates size classes as affected by cultivation, Soil Biol. Biochem., 20, 777–786, 1988.

Hamilton, J. G., DeLucia, E. H., George, K., Naidu, S. L., Finzi, A. C., and Schlesinger, W. H.: Forest carbon balance under elevated CO_2, Oecologia, 131, 250–260, 2002.

Hoosbeek, M. R., Li, Y., and Scarascia-Mugnozza, G.: Free atmospheric CO_2 enrichment (FACE) increased labile and total carbon in the mineral soil of a short rotation Poplar plantation, Plant and Soil, 281(1–2), 247–254, 2006.

Hoosbeek, M. R., Lukac, M., Van Dam, D., Godbold, D. L., Velthorst, E. J., Biondi, F. A., Peressotti, A., Cotrufo, M. F., De Angelis, P., and Scarascia-Mugnozza, G.: More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): Cause of increased priming effect?, Global Biogeochem. Cycles, 18, GB1040, doi:10.1029/2003GB002127, 2004.

Houghton, R. A.: The contemporary carbon cycle, in: Biogeochemistry, edited by: Schlesinger,

W. H., Elsevier, 473–513, 2003.
Houghton, R. A., Davidson, E. A., and Woodwell, G. M.: Missing sinks, feedbacks, and understanding the role of terrestrial ecosystems in the global carbon balance, Global Biogeochem. Cycles, 12, 25–34, 1998.
Janssens, I. A., Freibauer, A., Ciais, P., Smith, P., Nabuurs, G. J., Folberth, G., Schlamadinger, B., Hutjes, R. W. A., Ceulemans, R., Schulze, E.-D., Valentini, R., and Dolman, A. J.: Europe's terrestrial biosphere absorbs 7 to 12% of Europen anthropogenic CO_2 emissions, Science, 300, 1538–1542, 2003.
Jastrow, J. D.: Soil aggregate formation and the accrual of particulate and mineral-associated organic matter, Soil Biol. Biochem., 28, 665–676, 1996.
Jastrow, J. D., Miller, R. M., Matamala, R., Norby, R. J., Boutton, T. W., Rice, C. W., and Owensby, C. E.: Elevated atmospheric carbon dioxide increases soil carbon, Global Change Biol., 11, 2057–2064, 2005.
Krull, E. S., Baldock, J. A., and Skjemstad, J. O.: Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover, Functional Plant Biology, 30, 207–222, 2003.
Liberloo, M., Calfapietra, C., Lukac, M., Godbold, D., Luo, Z. B., Polle, A., Hoosbeek, M. R., Kull, O., Marek, M., Raines, C., Taylor, G., Scarascia-Mugnozza, G., and Ceulemans, R.: Woody biomass production during the second rotation of a bio-energy *Populus* plantation increases in a future high CO_2 world, Global Change Biol., 12(6), 1094–1106, 2006.
Lichter, J., Barron, S. H., Bevacqua, C. E., Finzi, A. C., Irving, K. F., Stemmler, E. A., and Schlesinger, W. H.: Soil carbon sequestration and turnover in a pine forest after six years of atmospheric CO_2 enrichment, Ecology, 86(7), 1835–1847, 2005.
Lukac, M., Calfapietra, C., and Godbold, D. L.: Production, turnover and mycorrhizal colonization of root systems of three *Populus* species grown under elevated CO_2 (POPFACE), Global Change Biol., 9, 838–848, 2003.
Miglietta, F., Peressotti, A., Vaccari, F. P., Zaldei, A., De Angelis, P., and Scarascia-Mugnozza, G.: Free-air CO2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system, New Phytologist, 150, 465–476, 2001.
Norby, R. J., DeLucia, E. H., Gielen, B., Calfapietra, C., Giardina, C. P., King, J. S., Ledford, J., McCarthy, H. R., Moore, D. J. P., Ceulemans, R., De Angelis, P., Finzi, A. C., Karnosky, D. F., Kubiske, M. E., Lukac, M., Pregitzer, K. S., Scarascia-Mugnozza, G., Schlesinger, W. H., and Oren, R.: Forest response to elevated CO2 is conserved across a broad range of

productivity, Proc. Nat. Acad. Sci. USA, 102(50), 18 052–18 056, 2005.
Norby, R. J., Hanson, P. J., O'Neill, E. G., Tschaplinski, T. J., Weltzin, J. F., Hansen, R. A., Cheng, W., Wullschleger, S. D., Gunderson, C. A., Edwards, N. T., and Johnson, D. W.: Net primary productivity of a CO_2-enriched deciduous forest and the implications for carbon storage, Ecological Applications, 12(5), 1261–1266, 2002.
Oades, J. M.: Soil organic matter and structural stability: mechanisms and implications for management, Plant and Soil, 76, 319–337, 1984.
Oades, J. M.: The role of biology in the formation, stabilization and degradation of soil structure, Geoderma, 56, 377–400, 1993.
Prentice, I. C., Farquhar, G. D., Fasham, M. J. R., Goulden, M. L., Heimann, M., Jaramillo, V. J., Kheshgi, H. S., Le Quere, C., Scholes, R. J., and Wallace, D. W. R.: The carbon cycle and atmospheric carbon dioxide, in: Climate Change 2001: The scientific basis, IPCC, Cambridge University Press, Cambridge and New York, 183–237, 2001.
Puget, P., Chenu, C., and Balesdent, J.: Total and yound organic matterdistributions in aggregates of silty cultivated soils, European Journal of Soil Science, 46, 449–459, 1995.
Scarascia-Mugnozza, G., Calfapietra, C., Ceulemans, R., Gielen, B., Cotrufo, M. F., DeAngelis, P., Godbold, D., Hoosbeek, M. R., Kull, O., Lukac, M., Marek, M., Miglietta, F., Polle, A., Raines, C., Sabatti, M., Anselmi, N., and Taylor, G.: Responses to elevated CO_2 of a short rotation, multispecies poplar plantation, in: Managed Ecosystems and CO_2, Springer Verlag, Berlin, 2006.
Schlesinger, W. H. and Lichter, J.: Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2, Nature, 411, 466–469, 2001.
Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S. J., Gregorich, E. G., Paul, E. A., and Paustian, K.: Measuring and understanding carbon storage in afforested soils by physical fractionation, Soil Sci. Soc. A. J., 66, 1981–1987, 2002.
Six, J., Carpentier, A., Van Kessel, C., Merckx, R., Harris, D., Horwath, W. R., and Lüscher, A.: Impact of elevated CO2 on soil organic matter dynamics as related to changes in aggregate turnover and residue quality, Plant and Soil, 234, 27–36, 2001.
Six, J., Elliott, E. T., and Paustian, K.: Aggregate and soil organic matter dynamics under conventional and no-tillage systems, Soil Sci. Soc. A. J., 63, 1350–1358, 1999.
Six, J., Elliott, E. T., and Paustian, K.: Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture, Soil Biol. Biochem., 32, 2099–2103, 2000.

Six, J., Elliott, E. T., Paustian, K., and Doran, J. W.: Aggregation and soil organic matter accumulation in cultivated and native grassland soils, Soil Sci. Soc. Am. J., 62, 1367–1377, 1998.

Tisdall, J. M. and Oades, J. M.: Organic matter and water-stable aggregates in soils, J. Soil Sci., 33, 141–163, 1982.

Van Doesburg, J. D. J.: Particle-size analysis and mineralogical analysis, In: Manual for soil and water analysis, edited by: Buurman, P., Van Lagen, B., and Velthorst E. J., Backhuys Publishers, Leiden, The Netherlands, 1996.

Van Lagen, B.: Soil Analyses, in: Manual for soil and water analyses, edited by: Buurman, P., Van Lagen, B., and Velthorst, E. J., Backhuys Publishers, Leiden, The Netherlands, 1996.

Van Veen, J. A. and Kuikman, P. J.: Soil structural aspects of decomposition of organic matter, Biogeochem., 11, 213–233, 1990.

Table 1. Carbon and nitrogen weight percentages of the mineral top soil in the sampled rings (1–4), October 2003.

Treatment			C %		N %		Bulk density (g soil · cm^{-3})	
		n	mean	s.e.	mean	s.e.	mean	s.e.
CO_2	Ambient	12	1.18	0.04	0.11	0.00	1.26	0.02
	FACE	12	1.20	0.03	0.13	0.01	1.17	0.04
N	Ambient	12	1.20	0.04	0.12	0.01	1.21	0.03
	Fertilized	12	1.18	0.03	0.12	0.00	1.23	0.04
Species	*Alba*	8	1.17	0.04	0.12	0.01	1.20	0.05
	Nigra	8	1.17	0.04	0.12	0.01	1.22	0.05
	Euramericana	8	1.23	0.05	0.13	0.01	1.23	0.04

Table 2. Effect of FACE, N-fertilization and species on sand free aggregate weight fractions.

Treatment			Aggregate weight fractions							
			53–250 μm		250–1000 μm		1000–2000 μm		>2000 μm	
		n	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
CO_2	Ambient	12	0.09	0.01	0.33	0.02	0.15	0.01	0.22	0.02
	FACE	12	0.09	0.01	0.33	0.02	0.15	0.01	0.24	0.03
N	Ambient	12	0.09	0.01	0.31	0.02	0.15	0.01	0.24	0.02
	Fertilized	12	0.09	0.01	0.35	0.02	0.15	0.01	0.22	0.03
Species	*Alba*	8	0.08	0.01	0.28	0.03	0.17	0.01	0.27	0.03
	Nigra	8	0.08	0.01	0.31	0.01	0.15	0.01	0.26	0.02
	Euramericana	8	0.10[a]	0.01	0.40[b]	0.02	0.13[c]	0.01	0.16[d]	0.03

[a] Significant species effect (P=0.032) on 53–250 μm aggregate weight fraction.
[b] Significant species effect (P=0.004) on 250–1000 μm aggregate weight fraction.
[c] Possible species effect (P=0.106) on 1000–2000 μm aggregate weight fraction.
[d] Significant species effect (P=0.043) on >2000 μm aggregate weight fraction.

Table 3. Effect of FACE, N-fertilization and species on sand free aggregate C fractions.

Treatment			Aggregate C fractions							
			53–250 μm		250–1000 μm		1000–2000 μm		>2000 μm	
		n	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
CO_2	Ambient	12	0.13	0.01	0.46	0.03	0.18	0.01	0.26	0.03
	FACE	12	0.11	0.01	0.42	0.03	0.19	0.01	0.29	0.04
N	Ambient	12	0.12	0.01	0.41	0.03	0.18	0.01	0.29	0.03
	Fertilized	12	0.12	0.01	0.47	0.03	0.19	0.01	0.26	0.03
Species	*Alba*	8	0.11	0.01	0.39	0.04	0.20	0.02	0.31	0.04
	Nigra	8	0.12	0.01	0.42	0.03	0.19	0.01	0.32	0.03
	Euramericana	8	0.14	0.01	0.51[a]	0.03	0.16	0.01	0.19[b]	0.03

[a] Possible species effect (P=0.069) on 250–1000 μm aggregate carbon fraction.
[b] Possible species effect (P=0.057) on >2000 μm aggregate carbon fraction.

Table 4. Effect of FACE, N-fertilization and species on sand free aggregate N fractions.

Treatment		Aggregate N fractions								
			53–250 μm		250–1000 μm		1000–2000 μm		>2000 μm	
		n	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
CO_2	Ambient	12	0.09	0.01	0.32	0.02	0.15	0.01	0.23	0.04
	FACE	12	0.09	0.01	0.30	0.02	0.14	0.01	0.22	0.03
N	Ambient	12	0.09	0.01	0.32	0.02	0.14	0.01	0.24	0.03
	Fertilized	12	0.08	0.01	0.31	0.02	0.15	0.01	0.22	0.03
Species	*Alba*	8	0.08	0.01	0.30	0.03	0.17	0.01	0.27	0.04
	Nigra	8	0.10	0.01	0.33	0.03	0.17	0.01	0.29	0.03
	Euramericana	8	0.08	0.01	0.31	0.03	0.10[a]	0.01	0.12[b]	0.01

[a] Significant species effect (P=0.009) on 1000–2000 μm aggregate nitrogen fraction.
[b] Significant species effect (P=0.006) on >2000 μm aggregate nitrogen fraction.

Table 5. Isolated iM-micro-aggregate weight, C and N fractions.

Treatment			iM-micro-aggregate weight fraction		C fraction		N fraction	
		n	mean	s.e.	mean	s.e.	mean	s.e.
CO_2	Ambient	12	0.53	0.01	0.63	0.01	0.73	0.06
	FACE	12	0.60[a]	0.03	0.71[b]	0.02	0.75	0.03
N	Ambient	12	0.57	0.03	0.66	0.02	0.70	0.05
	Fertilized	12	0.57	0.02	0.67	0.02	0.78	0.04
Species	*Alba*	8	0.56	0.03	0.68	0.03	0.68	0.05
	Nigra	8	0.56	0.03	0.66	0.02	0.66	0.04
	Euramericana	8	0.58	0.04	0.67	0.02	0.87[c]	0.05

[a] Possible CO_2 treatment effect (P=0.104) on iM-micro-aggregate weight fraction.
[b] Significant CO_2 treatment effect (P=0.003) on iM-micro-aggregate C fraction.
[c] Significant species effect (P=0.020) on iM-micro-aggregate N fraction.